amino acid position 100; wherein the anti-CD22 antibody is a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and has 90% or greater of the binding affinity of the RFB4 ds(Fv).

D5

12. (twice amended) The expression cassette of claim 11, wherein said antibody is an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

SUB #1

D6

16. (once amended) The expression cassette of claim 12, further comprising a sequence encoding for a linker peptide having the sequence of SEQ ID NO:5.

D7

27. (twice amended) The method of claim 22, wherein said immunoconjugate comprises an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

REMARKS

With entry of the instant amendment, claims 1, 5, 8, 9, 11, 12, 16, and 27 have been amended and claims 6, 15, 28, and 40-49 have been canceled. Accordingly, claims 1-5, 7-14, 16-27, and 29-39 are pending in the application. For convenience, a clean copy of the currently pending claims is provided in Appendix B attached hereto.

The amendments to the claims add no new matter and are supported throughout the application.

Claims 1 and 11 have been amended to recite an anti-CD22 antibody that is a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and has 90% or greater of the binding affinity of the RFB4 ds(Fv). Support for the amendment can be found in the specification, *e.g.*, at page 15, lines 10-14.

For convenience, the rejections will be addressed in the order presented in the July 5, 2001 Office Action.

Applicants thank the Examiner for the interview on December 18, 2001.

Rejections under 35 U.S.C. §103

Claims 1-17, 22-32 and 40-49 were rejected as allegedly obvious over Ghetie *et al.* in view of Reiter *et al.* and Kuan *et al.*

Applicants note that claims 40-49 have been canceled. Accordingly, the rejection is moot with regard to those claims.

The rejection alleges that it would have been obvious to one of skill in the art to obtain the nucleic acid and amino acid sequence of the RFB4 V_H and V_L regions, because the hybridoma, in order to make the conjugates as taught by Reiter *et al.* and Kuan *et al.*

Applicants respectfully disagree. The rejection contends that the V_H and V_L sequences can be obtained using primers and techniques well known to those of skill in the art. However, the Federal Circuit has held that a nucleic acid sequence is not obvious over general methods of isolating cDNA or DNA molecules *See, e.g., In re Deuel*, 34 USPQ2d, 1210, which states that

> Existence of general method of isolating cDNA or DNA molecules is essentially irrelevant to question of whether

> specific molecules themselves would have been obvious, in absence of other prior art that suggests claimed DNAs, nor does fact that general process can be conceived in advance for preparing undefined compounds mean that claimed specific compounds was precisely envisioned and therefore obvious

The nucleic acid sequence determination of a V_H or V_L is analogous to the sequencing of other nucleic acids using general methodology. For example, sequencing typically involves subcloning a nucleic acid fragment encoding a protein into a vector and using known primers corresponding to the vector sequence or primers corresponding to known sequence in the fragment to obtain the sequence information. Thus, existence of a method of obtaining a sequence of an antibody does not render obvious the sequence of the antibody. Accordingly, the claimed compositions and methods are unobvious over the cited art.

In addition, Applicants submit that the cited art does not make obvious dsFv immunoconjugates with the binding properties and cytotoxicity of the claimed RFB4dsFv immunoconjugates. As shown in the examples, an RFB49dsFv immunoconjugate of the invention exhibited binding properties that were comparable to that of the native RFB4 IgG (Example 2) and further, was more cytotoxic than a corresponding RFB4 scFv (Example 4). The art does not predict which dsFv immunoconjugates would have these characteristics. For example, Reiter *et al.*, in *Nature Biotechnology* 14:1239-1246, 1996, reference BE in the IDS submitted herewith, compare the binding of eight dsFv immunoconjugates. The binding affinity of the dsFv conjugates relative to the native Ig varied tremendously (Table 3). For example, the B3 and B1 antibody conjugates exhibited very poor binding properties relative to the native IgG, in contrast to dsFvRFB4. The authors concluded that their studies revealed that "each Fv is influenced differently in binding affinity to antigen after disulfide stabilization." (page 1242, column 2, paragraph 5, lines 8-9). Thus, various parameters appear to influence the binding properties of dsFv immunotoxins, and the art cannot predict

which conjugates will have the superior cytotoxicity and binding properties of a ds(Fv)RFB4 immunoconjugate of the invention.

Accordingly, the claimed immunoconjugates are unobvious over the cited. art. Applicants therefore request withdrawal of the rejection.

Rejections under 35 U.S.C. 112, second paragraph

Claims 42-47 were rejected as allegedly indefinite. The claims at issue have been canceled. Accordingly, the rejection is moot.

Rejections under 35 U.S.C. 112, first paragraph

Claims 5,6, 8-10, 12, 15, 16, 27, 28, and 44 were rejected as allegedly lacking enablement. The rejection alleges that the claims are not enabled because defining an epitope is not easy and further, that 90% sequence identity between two sequences has no common meaning within the art.

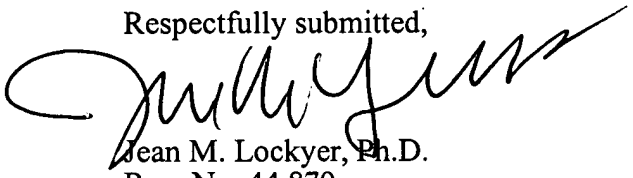
Applicants first note that claims 6, 15, and 28 have been canceled without prejudice. Accordingly, the rejection is moot with regard to those claims.

Further, Applicants respectfully disagree with the Examiner's contention that an antibody that binds to the same epitope as an RFB4dsFV requires extensive mapping in order to be identified. Indeed, it was well known in the art at the time the invention was made that epitopes could be mapped using a number of techniques including steric competition assays (*see, e.g., Antibodies: A Laboratory Manual*, Harlow & Lane, Eds, (1988) p. 590, which is cited at page 10, lines 21-29 and incorporated by reference into the specification). However, in order to expedite prosecution, the claims have been amended to recite a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) and 90% or greater of the binding affinity for the RFB4 dsFv. Applicants therefore request withdrawal of the rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If the Examiner believes a telephone conference would aid in the prosecution of this case in any way, please call the undersigned at 415-576-0200.

Respectfully submitted,



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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (twice amended) A recombinant immunoconjugate, comprising a therapeutic agent or a detectable label covalently linked to a recombinant antibody that binds an extracellular epitope of CD22 (an "anti-CD22 antibody") having a V_H with a cysteine at amino acid position 44 and a V_L with a cysteine at amino acid position 100; wherein the anti-CD22 antibody is a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and has 90% or greater of the binding affinity as the RFB4 ds(Fv).

5. (twice amended) The recombinant immunoconjugate of claim 1, wherein said anti-CD22 antibody is [a binding fragment that binds a same epitope as] an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, [wherein] in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, [wherein] in which a Cys residue is substituted for Gly at position 100.

8. (twice amended) The recombinant immunoconjugate of claim [6] 5, wherein said V_H chain is covalently linked to said V_L chain through a linker peptide.

9. (once amended) The recombinant immunoconjugate of claim [6] 5, wherein said V_H chain is linked to said V_L chain through a cysteine-cysteine disulfide bond.

11. (twice amended) An expression cassette encoding a recombinant immunoconjugate comprising a sequence encoding for a toxin peptide and an antibody that binds to an extracellular epitope of CD22 (an "anti-CD22" antibody) having a V_H encoding for a cysteine at amino acid position 44 and a V_L encoding for a cysteine at amino acid position 100; wherein the anti-CD22 antibody is a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and has 90% or greater of the binding affinity of the RFB4 ds(Fv).

12. (twice amended) The expression cassette of claim 11, wherein said antibody is [a binding fragment that binds to a same epitope as] an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, [wherein] in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, [wherein] in which a Cys residue is substituted for Gly at position 100.

16. (once amended) The expression cassette of claim [15] 12, further comprising a sequence encoding for a linker peptide having the sequence of SEQ ID NO:5.

27. (twice amended) The method of claim 22, wherein said immunoconjugate comprises [an antibody binding fragment that binds to a same epitope as] an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, [wherein] in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, [wherein] in which a Cys residue is substituted for Gly at position 100.

APPENDIX B
ALL CURRENTLY PENDING CLAIMS

1. (twice amended) A recombinant immunoconjugate, comprising a therapeutic agent or a detectable label covalently linked to a recombinant antibody that binds an extracellular epitope of CD22 (an "anti-CD22 antibody") having a V_H with a cysteine at amino acid position 44 and a V_L with a cysteine at amino acid position 100; wherein the anti-CD22 antibody is a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and has 90% or greater of the binding affinity of the RFB4 ds(Fv)..

2. (as filed) The recombinant immunoconjugate of claim 1, wherein said therapeutic agent is a toxin.

3. (as filed) The recombinant immunoconjugate of claim 2, wherein said toxin is a *Pseudomonas* exotoxin (PE) or a cytotoxic fragment thereof.

4. (as filed) The recombinant immunoconjugate of claim 3, wherein said cytotoxic fragment is PE38.

5. (twice amended) The recombinant immunoconjugate of claim 1, wherein said anti-CD22 antibody is an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

6. (canceled) The recombinant immunoconjugate of claim 1, wherein said antibody comprises a variable heavy (V_H) chain at least 90% identical to that set out in SEQ ID NO:2 over a comparison window of 10 amino acids, and a variable light (V_L) chain at least 90% identical to that set out in SEQ ID NO:4 over a

comparison window of 10 amino acids; and further, wherein said antibody binds to the same epitope as an RFB4 antibody comprising a V_H chain of SEQ ID NO:2 and a V_L chain of SEQ ID NO:4.

7. (once amended) The recombinant immunoconjugate of claim 3, wherein said variable heavy (V_H) chain is covalently linked to the carboxyl terminus of said toxin.

8. (twice amended) The recombinant immunoconjugate of claim 5, wherein said V_H chain is covalently linked to said V_L chain through a linker peptide.

9. (once amended) The recombinant immunoconjugate of claim 5, wherein said V_H chain is linked to said V_L chain through a cysteine-cysteine disulfide bond.

10. (as filed) The recombinant immunoconjugate of claim 8, wherein said linker peptide has the sequence of SEQ ID NO:5.

11. (twice amended) An expression cassette encoding a recombinant immunoconjugate comprising a sequence encoding for a toxin peptide and an antibody that binds to an extracellular epitope of CD22 (an "anti-CD22" antibody) having a V_H encoding for a cysteine at amino acid position 44 and a V_L encoding for a cysteine at amino acid position 100; wherein the anti-CD22 antibody is a binding fragment that competes for binding to a same epitope as an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100, and has 90% or greater of the binding affinity of the RFB4 ds(Fv).

12. (twice amended) The expression cassette of claim 11, wherein said antibody is an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position

44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

13. (as filed) The expression cassette of claim 11, wherein said toxin is a *Pseudomonas* exotoxin (PE) or a cytotoxic fragment thereof.

14. (as filed) The expression cassette of claim 11, wherein said cytotoxic fragment is PE38.

15. (canceled) The expression cassette of claim 11, wherein said antibody comprises a variable heavy (V_H) chain at least 90% identical to that set out in SEQ ID NO:2 over a comparison window of 10 amino acids, and a variable light (V_L) chain at least 90% identical to that set out in SEQ ID NO:4 over a comparison window of 10 amino acids; and further, wherein said antibody binds to the same epitope as an RFB4 dsFV as set out in claim 12.

16. (once amended) The expression cassette of claim 12, further comprising a sequence encoding for a linker peptide having the sequence of SEQ ID NO:5.

17. (as filed) A host cell comprising an expression cassette of claim 11.

18. (canceled) A V_H sequence substantially similar to that of SEQ ID NO:2.

19. (canceled) A V_L sequence substantially similar to that of SEQ ID NO:4.

20. (canceled) A nucleic acid sequence substantially similar to that of SEQ ID NO:1.

21. (canceled) A nucleic acid sequence substantially similar to that of SEQ ID NO:3.

22. (once amended) A method for inhibiting the growth of a malignant B-cell that expresses a CD22 molecule on the surface of the cell, said method comprising:

contacting said malignant B-cell with an effective amount of a recombinant immunoconjugate of claim 1, thereby inhibiting the growth of the malignant B-cell.

23. (as filed) The method of claim 22, wherein said toxin is a *Pseudomonas* exotoxin (PE) or a cytotoxic fragment thereof.

24. (as filed) The method of claim 22, wherein said malignant B-cell is contacted *in vivo*.

25. (as filed) The method of claim 22, wherein said malignant B-cell is selected from the group consisting of: a rodent B-cell, a canine B-cell, and a primate B-cell.

26. (as filed) The method of claim 23, wherein said cytotoxic fragment is a PE38 fragment.

27. (twice amended) The method of claim 22, wherein said immunoconjugate comprises an RFB4 disulfide-stabilized Fv (dsFv) comprising a variable heavy (V_H) chain as set out in SEQ ID NO:2, in which a Cys residue is substituted for Arg at position 44; and a variable light (V_L) chain as set out in SEQ ID NO:4, in which a Cys residue is substituted for Gly at position 100.

28. (canceled) The method of claim 22, wherein said immunoconjugate comprises an antibody comprising a variable heavy (V_H) chain at least 90% identical to that set out in SEQ ID NO:2 over a comparison window of 10 amino acids, and a variable light (V_L) chain at least 90% identical to that set out in SEQ ID NO:4 over a comparison window of 10 amino acids; and further, wherein said antibody binds to the same epitope as an RFB4 antibody comprising a V_H chain of SEQ ID NO:2 and a V_L chain of SEQ ID NO:4.

29. (amended) The method of claim 23, wherein a variable heavy chain is covalently linked at the carboxyl terminus of said toxin.

30. (amended) The method of claim 29, wherein said V_H chain is covalently linked to said V_L chain through a linker peptide.

31. (as filed) The method of claim 29, wherein said V_H chain is linked to said V_L chain through a cysteine-cysteine disulfide bond.

32. (as filed) The method of claim 31, wherein said linker peptide has the sequence of SEQ ID NO:5.

40. (canceled) An isolated nucleic acid encoding a V_H chain comprising an amino acid sequence as set out in SEQ ID NO:2.

41. (canceled) An isolated nucleic acid encoding a V_L chain comprising an amino acid sequence as set out in SEQ ID NO:4.

42. (canceled) An isolated nucleic acid encoding a V_H chain comprising a conservatively modified variant of an amino acid sequence set forth in SEQ ID NO:2.

43. (canceled) An isolated nucleic acid encoding a V_L chain comprising a conservatively modified variant of an amino acid sequence set forth in SEQ ID NO:4.

44. (canceled) An antibody that binds to an extracellular epitope of CD22 (an "anti-CD22 antibody") comprising a variable heavy (V_H) chain that is a conservatively modified variant of SEQ ID NO:2 and a variable light (V_L) chain that is a conservatively modified variant of SEQ ID NO:4, wherein the antibody binds to the same epitope as an RFB4 antibody comprising a V_H chain of SEQ ID NO:2 and a V_L chain of SEQ ID NO:4.

45. (canceled) The anti-CD22 antibody of claim 44, wherein said antibody is detectably labeled.

46. (canceled) The antibody of claim 44, wherein said antibody is conjugated to a therapeutic agent.

47. (canceled The antibody of claim 46, wherein said therapeutic agent is a *Pseudomonas* exotoxin (PE) or cytotoxic fragment thereof.

48. (canceled) A method for detecting the presence of CD22 protein in a biological sample, said method comprising:

(a) contacting said biological sample with an anti-CD22 antibody of claim 44;

(b) binding said antibody to said CD22 protein under immunologically reactive conditions to form an antibody-CD22 protein complex, wherein detection of said complex indicates the presence of said CD22 protein.

49. (canceled) The method of claim 48, wherein said antibody is detectably labeled.